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Expression of Recombinant Rat Interleukin-13 (IL-13) and Generation of a Neutralizing Rat IL-13 Antiserum

Fadi G. Lakkis,[1] Eddie N. Cruet, George M. Nassar,[2] Kamal F. Badr, and David W. Pascual*
*Renal Division, Emory University School of Medicine and Veterans Affairs Medical Center, Atlanta, Georgia 30033; and *Veterinary Molecular Biology, Montana State University, Bozeman, Montana 59717-3610*

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Using baculoviral and bacterial systems, we expressed biologically active recombinant rat IL-13 and generated neutralizing rat IL-13 antiserum. Recombinant rat IL-13 produced by baculovirus-infected insect cells stimulated proliferation of TF-1 premyeloid cell line and induced expression of 15-lipoxygenase mRNA in human peripheral blood monocytes. Antiserum generated by immunizing a rabbit with recombinant bacterial rat IL-13 specifically inhibited TF-1 proliferation induced by baculoviral rat IL-13 but did not neutralize human IL-13 mitogenic activity. Western blotting with anti-rat IL-13 serum revealed a ~12 kD protein band in supernatants of insect cells infected with recombinant baculovirus carrying the rat IL-13 cDNA. The availability of recombinant rat IL-13 and rat IL-13 antibodies should facilitate studying the role of IL-13 in rat models of human inflammatory disorders. © 1997 Academic Press

IL-13 is an immunomodulatory cytokine produced by T helper subset 2 (Th2) lymphocytes (1). Like IL-4, IL-13 directs synthesis of IgE by B lymphocytes and downregulates the inflammatory functions of monocytes and macrophages (1–3). Human or murine monocytes cultured in the presence of IL-13 display reduced ability to produce IL-1α, IL-1β, IL-6, IL-8, tumor necrosis factor (TNF) and nitric oxide (4,5). On the other hand, IL-13 stimulates IL-1 receptor antagonist (IL-1RA) production (4,5) and induces *de novo* expression of monocyte 15-lipoxygenase (15-LO), an enzyme which catalyzes the formation of anti-inflammatory eicosanoids from arachidonic acid (6). Unlike IL-4, IL-13 does not directly alter T lymphocyte and natural killer cell functions (1–3). The macrophage-inactivating actions of IL-13 have been demonstrated *in vivo* suggesting that it may have a role in the treatment of inflammatory diseases (7).

Numerous models of human autoimmune and inflammatory disorders have been established in the rat. The genes for several rat cytokines have been cloned and the respective recombinant proteins synthesized (8–11). These reagents are essential for *in vitro* and *in vivo* immunological studies and for the generation of neutralizing antibodies to rat cytokines. Based on nucleic acid sequence homology between human and mouse IL-13, we isolated and characterized a rat IL-13 cDNA clone (12). The open reading frame codes for a 12,126 Da, 111 amino acid mature polypeptide which shares 63% and 79% homology with human and mouse IL-13, respectively. In this study, we report the expression of biologically active recombinant rat IL-13 protein and generation of neutralizing antiserum to rat IL-13.

METHODS

Cytokines. Recombinant human granulocyte macrophage-colony stimulating factor (GM-CSF) and recombinant human IL-13 were purchased from R&D systems (Minneapolis, MN).

Construction of recombinant baculovirus and production of rat IL-13 protein. Recombinant rat IL-13 was produced utilizing a baculovirus expression system MAXBAC (Invitrogen, San Diego, CA) (13) in which rat IL-13 cDNA (12) was subcloned into transfer vector pBlueBacIII (Invitrogen) and subsequently introduced downstream of the baculovirus polyhedrin gene promoter by homologous recombination. High titer (3×10^8 pfu/ml) recombinant baculovirus carrying rat IL-13 cDNA (BACratIL-13) was generated by repeated propagation in *Spodoptera frugiperda* (Sf9) cells grown in complete Grace's media (Invitrogen) at 28 °C. BACratIL-13 virus was stored at 4 °C until further use. Recombinant rat IL-13 protein was then expressed by infecting log-phase Sf9 insect cells (Invitrogen) grown in Ex-Cell 401 serum-free medium at 28 °C with BACratIL-13 virus [multiplicity of infection (MOI) = 5 – 10]. Forty eight hours later, culture

[1] To whom correspondence should be addressed at VAMC, Research 151N, 1670 Clairmont Road, Atlanta, GA 30033. Fax: (404)728-4851. E-mail: fglakki@emory.edu.
[2] Current address: Department of Internal Medicine, Medical College of Virginia, Richmond, VA 23298.
Abbreviations: BACratIL-13, recombinant baculovirus which contains the coding sequence for rat IL-13; 15-LO, 15-lipoxygenase; RT-PCR, reverse transcriptase-polymerase chain reaction; Sf9, insect cells susceptible to infection by baculoviruses; TF-1, cytokine-dependent human premyeloid cell line.

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medium was harvested after centrifugation (1000g × 10 min at 4 °C to remove cells followed by 48000g × 30 min at 4 °C to remove viral particles), aliquoted and stored at −70 °C until assayed for biological activity. Control medium was harvested from non-infected Sf9 cells grown under identical conditions.

TF-1 proliferation assay. TF-1 human premyeloid cell line (15) was propagated in complete RPMI medium (RPMI 1640 plus 10% FCS, L-glutamine, penicillin, and streptomycin) supplemented with GM-CSF (2 ng/ml) in a 5% CO_2, 37 °C incubator. TF-1 cells, rested for 48 hours in complete medium without GM-CSF, were seeded at 4×10^5 cells/well in 96-well plates. Serial 1:2 dilution of either BACratIL-13 infected or non-infected Sf9 culture medium was added. 42 hours later, cells were pulsed with tritiated thymidine (0.5 μCi/well) (Amersham, IL) and incubated for an additional 6 hours. Cells were then harvested on fiberglass filter paper using a PhD cell harvester (Cambridge, MA) and tritiated thymidine incorporation was measured in a scintillation counter (Beckman, IL). Thymidine uptake by unstimulated cells represented background proliferation and was subtracted from each experimental point. All dilutions were tested in quadruplicates. Data is presented as mean ± standard deviation.

Preparation of human peripheral blood monocytes. Fresh buffy coat packs were obtained from healthy volunteers (American Red Cross, Atlanta, GA) and subjected to Ficoll-Hypaque (Life Technologies, MD) density gradient centrifugation (6). The mononuclear cell layer was recovered, washed with Hank's balanced salt solution (HBSS) (Life Technologies), suspended at 1.5×10^7 cells/ml of complete RPMI medium, and adhered to 100mm polystyrene tissue culture plates for 3 hrs at 37 °C, 5% CO_2. Following removal of floating cells, adherent cell population was incubated in complete RPMI medium in the presence or absence of recombinant cytokines for 36 hours. More than 90% of adherent cells stained positive for monocyte nonspecific esterase.

RNA isolation and analysis. Total cellular RNA was purified by the Chomczynski method (16) using RNAzol reagent (Biotecx Laboratories, TX). 15-LO mRNA was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) × 30 cycles as previously described (6).

Production of antiserum to rat IL-13. Recombinant rat IL-13 used for antibody production was produced in a bacterial expression system (Qiagen, CA) (14). Rat IL-13 cDNA was subcloned into pQE30 expression plasmid (Qiagen Inc.). A 6× Histidine tag introduced at the amino-terminus of the protein allowed rapid partial purification of rat IL-13 from *E. coli* lysates under denaturing conditions (8M Guanidine Hydrochloride) using a Nickel-NTA resin affinity column as described by the manufacturer (Qiagen) (14). Rat IL-13 was then dialyzed against phosphate-buffered saline (PBS, pH7.4) prior to injection into rabbits. Female New Zealand white rabbits were immunized subcutaneously with 20 μg of recombinant rat IL-13 in complete Freund's adjuvant (Sigma, MO). Booster immunizations were administered at three-week intervals using 5 μg of recombinant rat IL-13 in incomplete Freund's adjuvant (Sigma). Rabbits were bled periodically to test for antibody titers to rat IL-13.

Western blotting. Proteins in the supernatant of Sf9 cells were electrophoretically separated on a 12% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane and probed with 1:500 dilution of rabbit anti-rat IL-13 antiserum according to standard procedure (6). After thorough washing, the protein band to which the antibody bound was visualized by an enhanced chemiluminescence (ECL) detection system (Amersham) using a peroxidase-labeled anti-rabbit IgG (Amersham).

RESULTS AND DISCUSSION

Rat IL-13 induces proliferation of TF-1 cell line. The human premyeloid cell line TF-1 proliferates in response to both human and murine IL-13 (1). To test for rat IL-13 biological activity, we examined the proliferative activity of culture medium supernatant of BACratIL-13-infected Sf9 cells. As shown in Figure 1A, serial dilutions of this medium induced dose-dependent proliferation of TF-1 cells while culture medium supernatant of non-infected Sf9 cells did not. Maximum ^{3}H-thymidine uptake by TF-1 cells in response to rat IL-13 was comparable to that induced by recombinant human IL-13 (Fig. 1B).

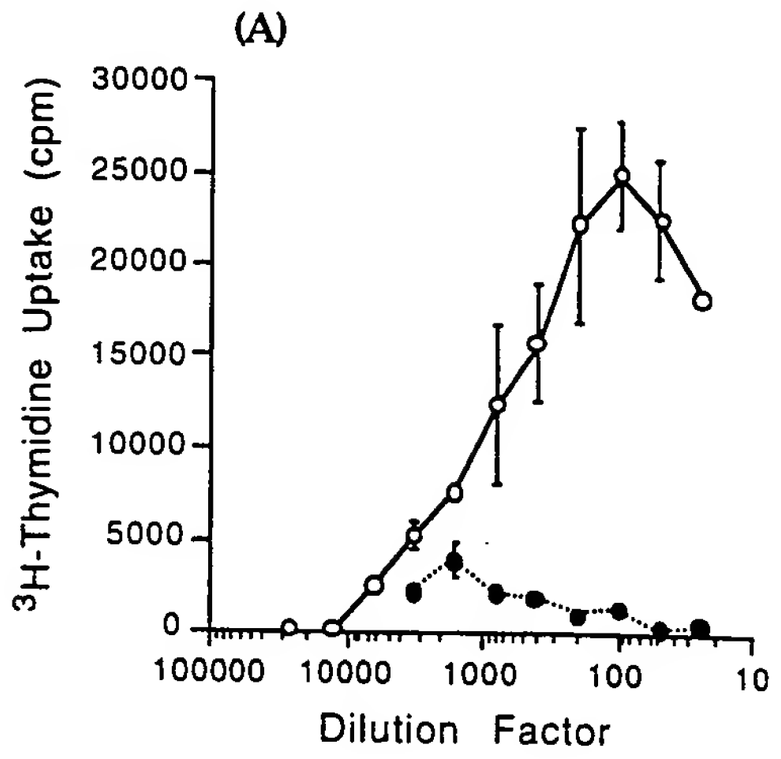

FIG. 1. (A) Biological activity of recombinant rat IL-13 measured in a TF-1 cell proliferation assay. Culture medium supernatant of BACratIL-13-infected Sf9 cells (*open circles*); culture medium supernatant of non-infected Sf9 cells (*solid circles*). (B) Biological activity of recombinant human IL-13 measured in a TF-1 cell proliferation assay.

Rat IL-13 induces 15-LO mRNA in human peripheral blood monocytes. Among T lymphocyte-derived cytokines, only IL-4 and IL-13 induce *de novo* expression of 15-LO in monocytes (6,17). To further confirm that BACratIL-13 virus expresses biologically active IL-13, we tested the ability of infected Sf9 cell culture medium supernatant to inducing 15-LO mRNA production in human peripheral blood monocytes. Figure 2 demon-

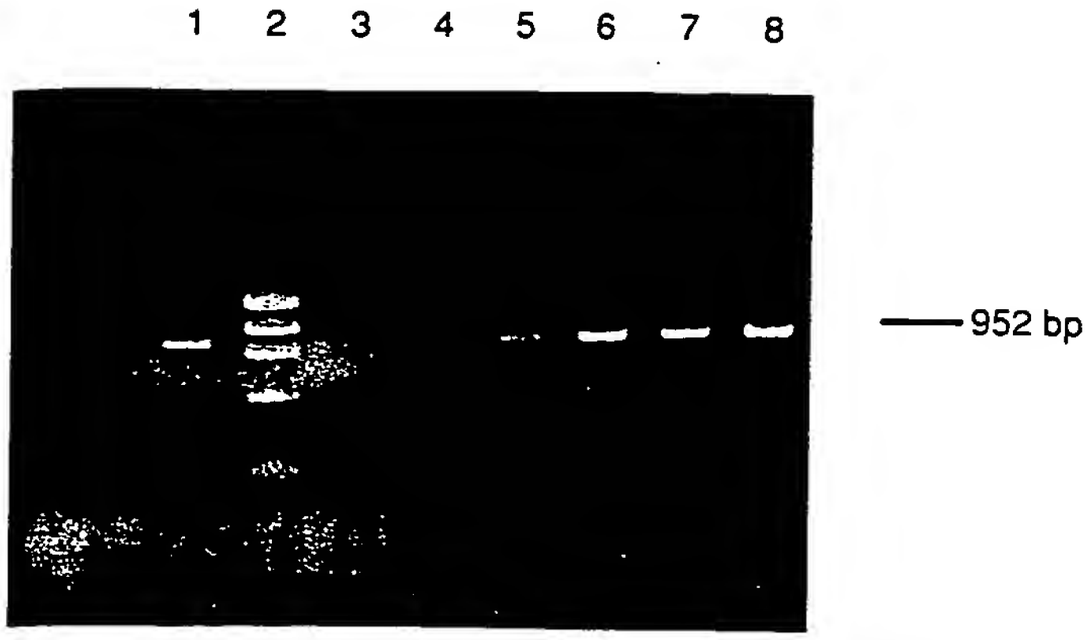

FIG. 2. Biological activity of recombinant rat IL-13 measured by induction of 15-LO mRNA in human monocytes (RT-PCR analysis). *Lane 1*, monocytes stimulated with human IL-13; *Lane 2*, DNA ladder (ϕX174RF DNA/*Hae*III fragments); *Lane 3*, monocytes stimulated with culture medium supernatant of non-infected Sf9 cells; *Lane 4–Lane 8*, monocytes stimulated with 10-fold decreasing dilutions of culture medium supernatant of BACratIL-13-infected Sf9 cultures (starting at 1:1 × 10^6 dilution in Lane 4).

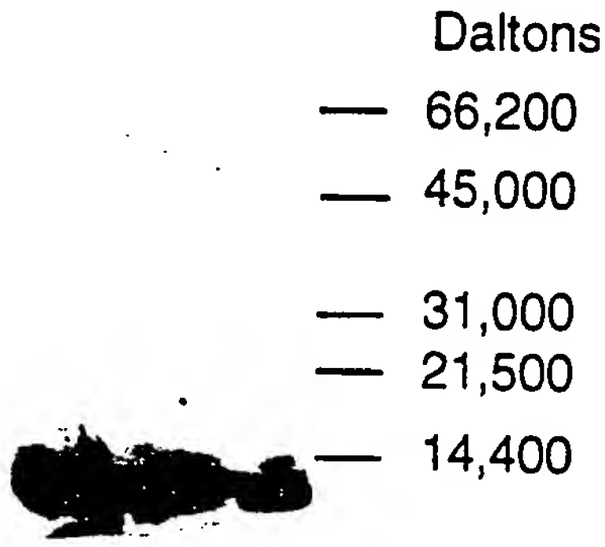

FIG. 4. Western blot analysis of rat IL-13 in culture medium supernatant of BACratIL-13-infected Sf9 cells. Media was collected and analyzed from three separate cultures.

strates dose dependent induction of 15-LO mRNA (952 bp RT-PCR product) by recombinant rat IL-13. As little as 1 : 1 × 10^6 dilution of culture medium supernatant of BACratIL-13 infected Sf9 cells induced 15-LO mRNA expression. Control supernatant obtained from non-infected Sf9 culture medium did not have an effect on 15-LO gene expression (Fig. 2, Lane 3).

Rabbit anti-rat IL-13 antiserum neutralizes rat IL-13 activity. As shown in Figure 3, serum obtained from a rabbit immunized with bacterial recombinant rat IL-13 specifically inhibited TF-1 cell proliferation induced by Sf9-produced rat IL-13 (solid bars). This antiserum did not neutralize human IL-13 activity (hatched bars). Moreover, irrelevant rabbit antiserum directed against adenovirus 5 (Ad5) did not suppress rat IL-13-induced proliferation of TF-1 cells (open bars). Enhanced proliferation of TF-1 cells at lower dilutions of Ad5 antiserum likely resulted from growth factors present in rabbit serum.

Detection of recombinant rat IL-13 by western blotting. Figure 4 demonstrates that antiserum generated against bacterial recombinant rat IL-13 detects a ~12 kD protein band in culture medium supernatants of BACratIL-13-infected Sf9 cells. This band corresponds to the predicted molecular weight of rat IL-13 (12,126 Da) (12) and was not detected in culture medium supernatant of non-infected Sf9 cells.

We described in this study the expression of biologically active recombinant rat IL-13 and generation of neutralizing rabbit anti-rat IL-13 serum. Recombinant rat IL-13 was active on human cells while the antiserum neutralized the rat cytokine only. We believe that these reagents are crucial for studying the

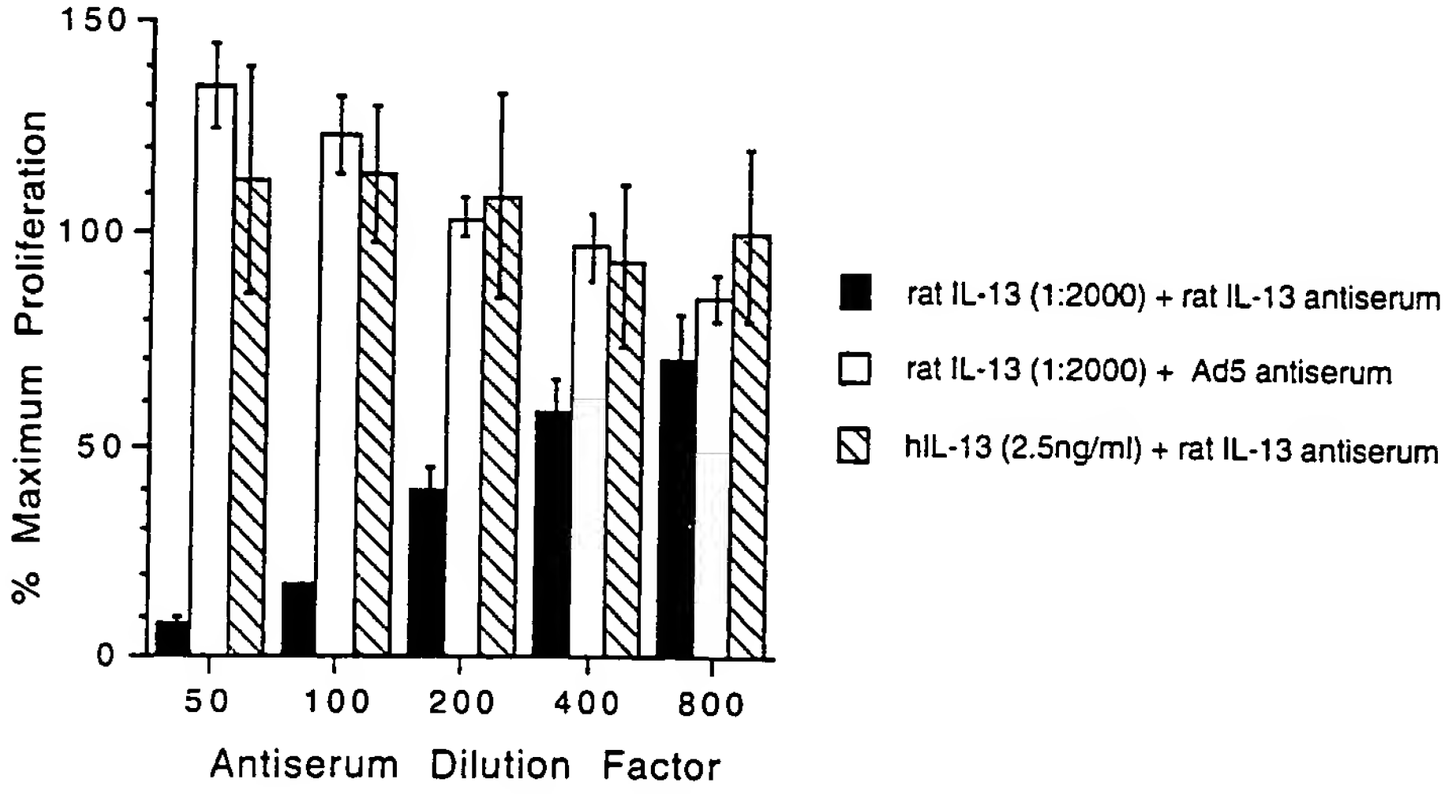

FIG. 3. Neutralization of recombinant rat IL-13 mitogenic activity by rabbit antiserum. Maximal proliferation = TF-1 cell proliferation induced by 1:2000 dilution of culture medium supernatant of BACratIL-13-infected Sf9 cells in the absence of antiserum.

role of IL-13 in rat models of immunologically-mediated disorders such as glomerulonephritis, arthritis, and encephalomyelitis. Furthermore, these reagents should facilitate the production of rat IL-13 monoclonal antibodies that are essential for *in vivo* neutralization studies and for quantitating rat IL-13 production in biological fluids.

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